

In the Claims

1. (currently amended) An isolated nucleic acid molecule that regulates the expression of a cold shock inducible gene under physiological conditions which cause the cold shock response in a bacterium, wherein said isolated nucleic acid molecule ~~is mediated by~~ mediates expression of the cold shock inducible gene, and wherein said isolated nucleic acid molecule is a portion of a 5'-UTR of the cold shock inducible gene or a substantially homologous sequence thereof.

Claim 2 (canceled)

3. (previously presented) The isolated nucleic acid molecule of Claim 1, wherein said 5'-UTR is a 5'-UTR of a cold-shock inducible gene selected from the group consisting of *cspA*, *cspB* and *csdA*.

Claim 4 (canceled)

5. (previously presented) The nucleic acid molecule of Claim 3, wherein said 5'-UTR comprises nucleotides +1 to +11 of the *cspA* 5'-UTR (nucleotides 1 to 11 of SEQ. ID. NO. 55) or a nucleotide sequence having substantial homology to nucleotides +1 to +11 of the *cspA* 5'-UTR (nucleotides 1 to 11 of SEQ. ID NO. 55).

6. (previously presented) The isolated nucleic acid molecule of Claim 1, wherein said cold shock inducible gene interacts with CspA protein.

7. (previously presented) An isolated nucleic acid molecule that represses the expression of a cold shock inducible gene under physiological conditions.

8. (previously presented) The isolated nucleic acid molecule of Claim 7, comprising at least a portion of the 5'-UTR of a cold shock inducible gene.

9. (previously presented) The isolated nucleic acid molecule of Claim 8, wherein said cold-shock inducible gene is selected from the group consisting of *cspA*, *cspB*, and *csdA*.

10. (previously presented) The nucleic acid molecule of Claim 8 wherein said cold-shock inducible gene comprises nucleotides +56 to +117 of the *cspA* 5'-UTR (nucleotides 56 to 117 of SEQ. ID. No. 55) or a nucleotide sequence having substantial homology to nucleotides +56 to +117 of the *cspA* 5'-UTR (nucleotides 56 to 117 of SEQ. ID. NO. 55).

11. (previously presented) A non-coding nucleic acid molecule that enhances the translation of a cold shock inducible gene under conditions that elicit the cold shock response of a bacterium.

12. (currently amended) The nucleic acid molecule of Claim 11, further comprising at least a portion of the 5'-UTR of a cold shock inducible gene.

13. (previously presented) The nucleic acid molecule of Claim 12 wherein said cold shock inducible gene is selected from the group consisting of *cspA*, *cspB*, and *csdA*.

14. (previously presented) The nucleic acid molecule of Claim 13, comprising nucleotides +123 to +135 of the *cspA* 5'-UTR (nucleotides 123 to 135 of SEQ. ID. NO. 55) or a nucleotide sequence having substantial homology to nucleotides +123 to +135 of the *cspA* 5'-UTR (nucleotides 123 to 135 of SEQ. ID. NO. 55).

15. (previously presented) The nucleic acid molecule of Claim 14 comprising a sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49, and SEQ ID NO:50.

16. (previously presented) A nucleic acid vector that enhances translation of a gene under conditions that elicit a cold-shock response in a bacterium comprising a downstream box and a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first

nucleic acid fragment and a first cold shock inducible gene, wherein said first nucleic acid fragment enhances translation of said first cold shock inducible gene under conditions that elicit the cold shock response in bacterium, and wherein said nucleic acid vector is free from said first cold shock inducible gene.

17. (previously presented) The nucleic acid vector of Claim 16 further comprising a Shine-Dalgarno sequence.

18. (original) A nucleic acid vector of Claim 16 further comprising a cold box, wherein said vector directs prolonged expression and enhances translation of a gene under conditions that elicit a cold shock response in a bacterium.

19. (previously presented) A nucleic acid vector that directs the prolonged expression and enhances the translation of a gene under conditions of physiological stress that elicit a cold shock response of a bacterium, and represses the expression of the gene under physiological conditions comprising a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, wherein said first nucleic acid fragment enhances translation of said first cold shock inducible gene under cold shock conditions, a second nucleic acid fragment derived from said first nucleic acid molecule or from a second nucleic acid molecule, said second nucleic acid molecule comprising said second nucleic acid fragment and a second cold shock inducible gene, wherein said second nucleic acid fragment represses expression of said first or second cold shock inducible gene under physiological conditions, a cold box, and a downstream box, wherein when said first nucleic acid fragment and said second nucleic acid fragment are derived from said first nucleic acid molecule, said vector is free from said first cold shock inducible gene, and wherein when said first and second nucleic acid fragments are respectively derived from said first and second nucleic acid

molecules, said vector may comprise one of said first cold shock inducible gene or said second cold shock inducible gene.

20. (previously presented) The vector of claim 16 further comprising a coding region of a second cold shock inducible gene.

21. (previously presented) The vector of Claim 18, further comprising a coding region of a second cold-shock inducible gene.

22. (previously presented) The vector of Claim 19, further comprising a coding region of a third cold-shock inducible gene, wherein when said first nucleic acid fragment is derived from said first nucleic acid molecule and said second nucleic acid fragment is derived from said second nucleic acid molecule, said third cold shock inducible gene may be one of said first cold shock inducible gene or said second cold shock inducible gene.

23. (previously presented) The vector of Claim 16 further comprising a coding region of a heterologous gene.

24. (previously presented) The vector of Claim 18 further comprising a coding region of a heterologous gene.

25. (previously presented) The vector of Claim 19, further comprising a coding region of a heterologous gene.

26. (previously presented) The vector of Claim 16, further comprising a promoter and at least one restriction site downstream of said first nucleic acid fragment and said downstream box for inserting an additional DNA fragment.

27. (previously presented) The vector of Claim 18, further comprising a promoter and at least one restriction site downstream of said cold box, said first nucleic acid fragment, and said downstream box for inserting an additional DNA fragment.

28. (previously presented) A nucleic acid vector that directs prolonged expression and enhances translation of a gene under conditions of physiological stress that elicit a cold shock response of a bacterium, and represses expression of the gene under physiological conditions comprising a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, wherein said first nucleic acid fragment enhances translation of said first cold shock inducible gene under cold shock conditions, a second nucleic acid fragment derived from said first nucleic acid molecule or from a second nucleic acid molecule, said second nucleic acid molecule comprising said second nucleic acid fragment and a second cold shock inducible gene, wherein said second nucleic acid fragment represses expression of said first or second cold shock inducible gene under physiological conditions, a cold box, a downstream box, a promoter and at least one restriction site downstream of said cold box, said first nucleic acid fragment, said second nucleic acid fragment, and said downstream box for inserting an additional DNA fragment.

29. (previously presented) The vector of claim 26, wherein said additional DNA fragment comprises a coding region of a second cold shock inducible gene.

30. (previously presented) The vector of Claim 27, wherein said additional DNA fragment comprises a coding region of a second cold shock inducible gene.

31. (previously presented) The vector of Claim 28, wherein said additional DNA fragment comprises a coding region of a cold shock inducible gene.

32. (previously presented) The vector of Claim 26, wherein said additional DNA fragment comprises a coding region of a heterologous gene.

33. (previously presented) The vector of Claim 27, wherein said additional DNA fragment comprises a coding region of a heterologous gene.

34. (previously presented) The vector of Claim 28, wherein said additional DNA fragment comprises a coding region of a heterologous gene.

35. (original) A transformed bacteria containing the vector of Claim 16.

36. (original) A transformed bacteria containing the vector of Claim 18.

37. (original) A transformed bacteria containing the vector of Claim 19.

38. (previously presented) A method for overexpressing a gene comprising the steps of: transforming bacteria with a nucleic acid vector that enhances translation of a gene under conditions that elicit a cold shock response in a bacterium comprising a downstream box, a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, and a gene, wherein said first nucleic acid fragment enhances translation of said first cold shock inducible gene under cold shock conditions, and subjecting said bacteria to conditions that elicit a cold shock response.

39. (previously presented) A method for overexpressing a gene comprising the steps of: transforming bacteria with a nucleic acid vector comprising a downstream box, a cold box, a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, and a gene, wherein said vector directs prolonged expression and enhances translation of a gene under conditions that elicit a cold shock response in a bacterium, and subjecting said bacteria to conditions that elicit a cold shock response.

40. (previously presented) A method for overexpressing a gene comprising the steps of: transforming bacteria with a nucleic acid vector comprising a first nucleic acid fragment derived from a first nucleic acid molecule comprising said first nucleic acid fragment and a first cold shock inducible gene, wherein said first nucleic acid fragment enhances translation of said

first cold shock inducible gene, a second nucleic acid fragment derived from said first nucleic acid molecule or from a second nucleic acid molecule, said second nucleic acid molecule comprising said second nucleic acid fragment and a second cold shock inducible gene, wherein said second nucleic acid fragment represses expression of said cold shock inducible gene, a cold box, a downstream box, and a gene, wherein said vector directs prolonged expression and enhances translation of a gene under conditions that elicit a cold shock response of a bacterium and represses expression of the gene under physiological conditions, and subjecting said bacteria to conditions that elicit a cold shock response.

41. (previously presented) The method of Claim 40, wherein said overexpression causes a reduction in the expression of at least one endogenous protein.

42. (original) The method of Claim 38, wherein said overexpression causes a reduction in the expression of at least one endogenous protein.

43. (original) The method of claim 39, wherein said overexpression causes a reduction in the expression of at least one endogenous protein.

44. (previously presented) The method of Claim 40, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

45. (original) The method of Claim 38, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

46. (original) The method of Claim 39, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

47. (original) The method of Claim 43, wherein said temperature is about 10-15°C.

48. (original) The method of Claim 44, wherein said temperature is about 10-15°C.

49. (original) The method of Claim 45, wherein said temperature is about 10-15°C.

50. (previously presented) A vector capable of expressing a heterologous gene in a bacterium at physiological temperature or under conditions that elicit a cold shock response comprising regulatory elements in the following order: a promoter, at least a portion of a 5'-UTR of a cold shock inducible gene, a Shine-Dalgarno sequence, a translation initiation codon, a downstream box, and at least one restriction enzyme recognition site for insertion of said heterologous gene.

51. (previously presented) The vector of Claim 50, further comprising an additional nucleic acid fragment inserted at said restriction enzyme recognition site, wherein said fragment comprises a coding region of a cold shock inducible gene and wherein said fragment is regulated by said regulatory elements.

52. (previously presented) The vector of Claim 50, further comprising an additional nucleic acid fragment inserted at said restriction enzyme site, wherein said fragment comprises a coding region of a heterologous gene and wherein said fragment is regulated by said regulatory elements.

53. (original) A transformed bacteria containing the vector of Claim 50.

54. (previously presented) A method of overexpressing a gene comprising transforming bacteria with a nucleic acid vector of Claim 51 or Claim 52 and subjecting said bacteria to conditions that elicit a cold shock response.

55. (original) The method of Claim 54, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

56. (original) The method of claim 55, wherein said temperature is about 10-15°C.

57. (currently amended) An isolated nucleic acid molecule ~~competent~~ competent to prolong the expression of a cold shock gene during abdendation of a bacterium to physiological



stress which elicits a cold shock response, said nucleic acid molecule comprising at least 8 of the first 25 nuclear tags of a 5'-UTR with a cold shock inducible mRNA transcript, and a promoter active under conditions of physiological stress to induce said cold shock response in said bacterium.